Growth and Ectomycorrhizal Development of Pine Seedlings in Nursery Soils Infested with the Fungal Symbiont Pisolithus tinctorius

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Abstract. Vegetative and basidiospore inocula of *Pisolithus tinctorius* were artificially introduced into soils of three nurseries in Georgia, Florida, and North Carolina. After one growing season in the North Carolina nursery, growth stimulation by *Pisolithus* ectomycorrhizae from vegetative inoculum was 140 percent on seedlings of *Pinus taeda* and approximately 100 percent on seedlings of *P. virginiana* and *P. strobus*. Vegetative inoculum of *Pisolithus* formed 97, 94, and 77 percent of the ectomycorrhizae on *P. taeda*, *P. virginiana*, and *P. strobus*, respectively. Ectomycorrhizae formed from basidiospore inoculum did not dominate the root systems or stimulate seedling growth as much as vegetative inoculum. In the Florida nursery, *Pisolithus* ectomycorrhizae produced by both types of inocula were in approximately even mixtures with ectomycorrhizae formed by naturally occurring fungi. Growth stimulation by *P. tinctorius* ectomycorrhizae was not observed on seedlings of *P. taeda*, *P. elliottii* var. *elliottii*, or *P. clausa* in this nursery. Ectomycorrhizal development by *P. tinctorius* on *P. taeda*, *P. virginiana*, and *P. clausa* did not occur in the Georgia nursery, probably due to ineffective soil fumigation. Plant-parasitic nematodes and high populations of *Pythium irregulare–P. debaryanum* were isolated from plots in this nursery. The practical significance of lateral spread of *Pisolithus* into noninfested soil adjacent to infested soil and basidiocarp production is discussed. These results show that *P. tinctorius* can be artificially introduced into fumigated soil and form specific ectomycorrhizae on various pine species under diverse cultural conditions used in conventional nursery production of pine seedlings. ***Forest Sci. 22:91–100.***

Additional key words. Nematode-*Pythium* complex, nursery cultural practices, forestation, pine seedling quality.

USING specific ectomycorrhizal fungi on tree seedlings to improve survival and growth in forestation programs has been frequently suggested, but only a few practical efforts have been made. In Austria, Moser (1958, 1959) showed that pure cultures of *Boletus plorans* artificially introduced into nursery soils formed ectomycorrhizae on *Pinus cembra* seedlings.

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These seedlings, when planted at high elevations near the timberline, were superior to pine seedlings without *B. plorans* ectomycorrhizae. On a more limited scale, other workers (Shemakhanova 1967, Theodorou and Bowen 1970, Theodorou 1971, Hacskaylo and Vozzo 1971, Lamb and Richards 1974) showed that ectomycorrhizae formed by pure cultures of specific fungi on tree seedlings under controlled conditions improved field performance of the seedlings.

Recently, mycelium of *Pisolithus tinctorius* (Pers.) Coker and Couch (Pt) was introduced into fumigated soil in microplots, formed ectomycorrhizae on over 90 percent of short roots on loblolly pine seedlings, and increased seedling growth by over 100 percent (Marx and Bryan 1975). Vegetative mycelium was superior to basidiospores of Pt because it formed more ectomycorrhizae and stimulated more seedling growth. Root colonization by Pt occurred in spite of high levels of airborne inocula of naturally occurring fungal symbionts, such as *Thelephora terrestris* Ehrh. ex Fr. Control seedlings had approximately 45 percent ectomycorrhizae formed by *T. terrestris*.

Pine seedlings "tailored" with Pt ectomycorrhizae can be expected to show increased tolerance to extremes of soil acidity, high sulfur and aluminum levels, and high soil temperatures (Marx and others 1970a, Marx and Bryan 1971, Schramm 1966). Pt is one of the few ectomycorrhizal fungi capable of surviving on roots of tree seedlings on drastically disturbed sites, such as strip-mined coal spoils (Schramm 1966). Seedlings with Pt ectomycorrhizae produced in large numbers in nurseries should be suitable for planting on adverse sites because of their ecological adaptability to harsh soil conditions. Seedlings with Pt ectomycorrhizae also grow faster than seedlings ectomycorrhizal with other fungi under good growth conditions (Marx and Bryan 1975), suggesting that Pt may be a desired ectomycorrhizal symbiont for pine seedlings planted on all sites.

The purpose of this research was to introduce Pt artificially into soils of three southern nurseries and to determine the value of Pt in stimulating growth of various species of pines in these nurseries.

Materials and Methods

Pisolithus tinctorius (isolate 55) was cultured in October 1972 from a basidiocarp under loblolly pine in Athens, Georgia. After 4 mo in vermiculite-peat moss-nutrient at 22°C, mycelium had colonized the substrate completely. The vegetative inoculum was removed from the containers, screened to a particle size of 0.5 cm, leached in tapwater and hand squeezed to remove excess water. Details of these procedures have been published (Marx and Bryan 1975). Six-liter volumes of vegetative inoculum were placed in large plastic bags and stored in insulated containers maintained between 5°C and 15°C during transport. Vegetative inoculum was stored for a maximum of 40 hr before use.

Basidiospores of Pt were removed from basidiocarps collected under pines on strip-mined coal wastes in Fabius, Alabama, and Slippery Rock, Pennsylvania,[1] and on strip-mined kaolin wastes in Gordon, Georgia. The three basidiospore collections were stored dry at 5°C in darkness for 5 to 8 mo prior to use. Basidiospores used for soil infestation were equal mixtures of the three collections. Five gm (approximately 5.5 billion spores) quantities of basidiospores were stored in plastic bags in an insulated ice chest prior to use.

Nine plots were installed in a nursery bed for each of three pine species at each of three nurseries. The plots, 1.2 m by 1.8 m, were spaced approximately 7 m apart. None were within 7 m of the end of a bed. The three ectomycorrhizal treatments were (1) vegetative mycelium of Pt in vermiculite-peat moss (VMI); (2) basidiospores of Pt (BI); and (3) noninoculated controls (NI). The experiment was a randomized complete design with three replications for each mycorrhizal treatment on three pine species at each nursery. Treatments were

[1] Basidiospores collected by Richard J. Medve, Department of Biology, Slippery Rock State College, Slippery Rock, Pennsylvania 16057.

applied as follows: (1) 6-liter volumes of VMI were broadcast evenly over a plot (about 2.8 liters inoculum per m^2 soil surface) and immediately mixed into the upper 10 to 12 cm of soil; (2) 5 gm of BI were mixed thoroughly with 6 liters of moist vermiculite-peat moss in large plastic bags, broadcast evenly over a plot (approximately 2.55×10^9 spores per m^2), and immediately mixed into the surface soil as above; and (3) 6-liter volumes of vermiculite-peat moss mixture without inoculum (NI) were mixed as above to each control plot. Basidiospores were mixed with moist vermiculite-peat carrier because water suspension of spores decreases their effectiveness as inoculum (Marx 1976).

The experiments were installed May 15–23, 1973. Information relevant to each nursery follows:

Morgan Memorial Nursery, Byron, Georgia.—Test species were the Choctowhatchee variety of sand pine [*Pinus clausa* (Chapm.) Vasey] from a Florida seed source, loblolly pine from a Georgia seed source, and Virginia pine (*P. virginiana* Mill.) from a southern Kentucky seed source. The sand pine nursery section was fumigated in April 1973 with 560 kg per hectare of Pestmaster (98 percent methyl bromide) under polyethylene. Virginia pine seedlings had been grown in this section the previous season. The loblolly pine section was similarly fumigated in October 1972. Corn had been grown as a cover crop the previous season. The Virginia pine section had been fumigated in October 1971 with Pestmaster, and sand pine was grown in 1972. This section was not fumigated in 1972 or 1973. Unfortunately soil moisture was too high and soil temperatures were too low for effective fumigation on all dates. Prior to installation of the experiment, 1680 kg per hectare of dried cow manure and 672 kg per hectare of 5-10-15 granular fertilizer were applied to all plots. The loblolly and Virginia pine plots were also treated with 2.2 kg per hectare diphenamid and 1.1 kg per hectare of prometryne herbicides. Chemical analyses[2] of soil from all plots before the experiment were similar (pH 5.9, and 187, 222, 1345, 44, 96, and 74 kg per hectare of available P, K, Ca, Mg, Mn, and NO_3, respectively). The soil type in all plots was sandy loam.

All seeds were treated with Arasan® and aluminum oxide prior to planting. Sand and Virginia pines were broadcast seeded and loblolly pine was row seeded, each to obtain approximately 325 seedlings per m^2. After seeding, all beds were pressed with a drum and hydromulched with 1680 kg per hectare of aspen fiber.

During the 1973 growing season, a total of 1950 liters per hectare of mineral spirits were sprayed periodically on all plots for weed control. The loblolly pine plots were also sprayed with a total of 84 kg per hectare of Fermate® (76 percent ferbam) between May and July to control *Cronartium fusiforme*. All plots also received three applications of 37 kg per hectare of NH_4NO_3 through the irrigation system.

M. D. Andrews Memorial Nursery, Chiefland, Florida.—Test species were the Ocala race of sand pine, loblolly pine, and slash pine (*P. elliottii* Engelm. var. *elliottii*) from Florida sources.

Cropping histories on all experimental plots for the three previous years were pine in 1970, corn with stubble plowed under in 1971, and fallow in 1972. In March 1973, 225 kg per hectare of 0-45-0 and 280 kg per hectare of elemental sulfur were added to soil in all nursery sections. Sulfur was added to acidify the soil because of alkaline irrigation water. All beds were fumigated under polyethylene in April 1973 with 672 kg per hectare of Nemaster (68 percent methyl bromide). Chemical analyses of soil from all plots before the experiment were similar (pH 4.8, and 169, 250, 760, 44, 19, and 41 kg per hectare of available P, K, Ca, Mg, Mn, and NO_3, respectively). Total N and organic matter were 0.056 and 0.11 percent, respectively.

[2] Done by the Soil Testing Laboratory, Cooperative Extension Service, University of Georgia, Athens.

The soil in all plots was loamy sand. All seeds were treated with Arasan®, latex sticker, and aluminum oxide. All pine species were row seeded for a density of 382 seedlings per m^2, and all beds were hydromulched with about 1350 kg per hectare of aspen fiber.

During the growth season all plots were fertilized through the irrigation system with two applications of 56 kg per hectare of NH_4NO_3 and sprayed periodically with a total of 907 liters per hectare of mineral spirits for weed control. The loblolly and slash pine plots were also sprayed with a total of 54 kg per hectare of Fermate® (76 percent ferbam) for fusiform rust control.

Ralph Edwards State Forest Nursery, Morganton, North Carolina.—Test species were eastern white pine (*P. strobus* L.), loblolly pine, and Virginia pine. The Virginia pine seed came from the nursery seed orchard; white and loblolly pine seeds were from North Carolina sources. The white pine nursery section was fumigated with 392 kg per hectare of Nemaster under polyethylene in October 1972. The loblolly and Virginia pine sections were fumigated similarly in April 1973. Pines were grown in all nursery sections in 1972. Prior to installation of the experiment, the loblolly and Virginia pine plots were fertilized with 336 kg per hectare and the white pine plots received 112 kg per hectare of NH_4NO_3. Chemical analyses of soil from plots before the experiment were similar (pH 5.4, and 88, 184, 684, 122, 66, and 241 kg per hectare of available P, K, Ca, Mg, Mn, and NO_3, respectively). Total N and organic matter were 0.09 and 1.46 percent, respectively. The soil in all plots was sandy clay loam.

Seeds were treated with Arasan® and aluminum oxide, then row sown for a density of 325 seedlings per m^2. After planting, beds were mulched with a 0.5 cm layer of 8- to 10-year-old pine-hardwood sawdust which was not fumigated.

During the 1973 growing season, the white pine plots were fertilized with 168 kg per hectare and the loblolly and Virginia pine plots received 112 kg per hectare of NH_4NO_3. All plots were sprayed periodically with a total of 912 liters per hectare of mineral spirits for weed control. Cygon (23.4 percent dimethoate) was applied at 0.87 liter per 238 liters of water per hectare to all plots in August. In September, all plots received 112 kg per hectare of KCl.

Plots in each nursery were examined several times during the growing season for ectomycorrhizal development and basidiocarp production by Pt and other ectomycorrhizal fungi. Seedlings were harvested after 6 months in Georgia and North Carolina and 8 months in Florida. All plots were undercut with a root pruning bar at a depth of 20 cm and seedlings were lifted by hand. Seedlings per plot were counted and 10 seedlings from each plot were chosen at random for measurements of stem height, stem diameter at root collar, and fresh weights of shoots and roots. Degree of ectomycorrhizal development was estimated visually. During evaluation, all ectomycorrhizae and nonmycorrhizal short roots on two or three seedlings per plot were counted. These counts differed by less than 10 percent from visual estimates of mycorrhizae on the same seedlings. Frequently, the distinctive ectomycorrhizae formed by Pt (Marx and Bryan 1975) occurred in mixtures with other fungal symbionts on the same root system. In these instances, after total ectomycorrhizal development was estimated, another estimate was made for those formed by Pt.

Seedlings at various distances and from both ends of the Pt plots were lifted and the roots examined to determine the amount of lateral spread of Pt into noninfested soil from the infested plots.

Five subsamples of soil were collected at random from each plot, combined, and stored in plastic bags at 10°C until assayed. Each sample was assayed for pythiaceous fungi using modified Kerr's medium (Hendrix and Kuhlman 1965) and the procedures of Marx (1973) within 4 hr of collection. A complete chemical analysis was also made on each sample. Within 96 hr

of collection, a 100 cm^3 volume of soil from each sample was assayed[3] for plant-parasitic nematodes by Jenkins' (1964) centrifugal-flotation technique. Lateral roots with 100 to 150 representative ectomycorrhizae formed by Pt were removed from seedlings grown in each plot of the Pt treatments. Within 2 hr of collection, the roots were washed, surface sterilized in 100 mg per liter of $HgCl_2$ for 2.5 min, rinsed in sterile water, and individually tubed on agar medium for possible reisolation of Pt (Marx and others 1970b). Analyses of variance were made on all data and differences among means were evaluated with Duncan's multiple range test.

Results

Morgan Nursery, Georgia.—After 3 mo of seedling growth, we suspected that soil fumigation had not been effective, especially in the loblolly pine plots. This suspicion was based on the early appearance of large quantities of basidiocarps of *Thelephora terrestris*. These were present on stems of nearly half of the loblolly pine seedlings, with numerous other basidiocarps present on the soil surface. Previous observations indicated that if soil fumigation was effective it would take *T. terrestris* at least 4 mo to recolonize soil and form sufficient ectomycorrhizae to support large numbers of basidiocarps. Ectomycorrhizae characteristic of *T. terrestris* (Marx and others 1970b) were present in abundance on the seedlings. Virginia pine seedlings in all plots after 3 mo exhibited stunting and chlorosis of foliage. Only a few ectomycorrhizae and basidiocarps formed by *T. terrestris* were found on these seedlings. Sand pine seedlings also had chlorotic foliage after 3 mo, but not to the extent found on Virginia pine seedlings. Basidiocarps of *Thelephora* were also evident in these plots.

After 6 mo there were no differences in seedling growth of any pine species due to Pt treatment. Only a few (< 1 percent) Pt ectomycorrhizae were found on the loblolly pine seedlings in the VMI treatment and none in the BI treatment. Attempts to reisolate Pt from these seedlings were abandoned because of the low incidence of ectomycorrhizae. Over 2000 plant-parasitic nematodes per liter of soil were extracted from the loblolly pine plots. Stunt (*Tylenchorhynchus claytoni* Steiner), spiral (*Helicotylenchus* spp.), and ring (*Criconemoides* spp.) nematodes occurred in approximate population ratios of 4 : 2 : 1. Also, an average of 51 propagules per gram of soil of *Pythium irregulare–P. debaryanum* (Hendrix and Campbell 1973) were isolated from the loblolly pine plots. Feeder root necrosis was evident on the majority of these seedlings. Ectomycorrhizal development varied between 54 and 81 percent and averaged 70 percent.

The Virginia pine plots contained over 1200 stunt nematodes per liter of soil and 30 propagules of *P. irregulare–P. debaryanum* per gram of soil. Most seedlings showed feeder root necrosis. Ectomycorrhizal development varied between 76 and 90 percent and averaged 85 percent. Seedlings from soil infested with VMI of Pt were slightly larger than those from the other two treatments. These seedlings had over 90 percent of their feeder roots ectomycorrhizal, but only 10 percent of these were formed by Pt. Pt was reisolated from over 35 percent of the surface-sterilized ectomycorrhizae. No Pt ectomycorrhizae were observed on seedlings from the BI plots.

Results from the sand pine seedlings were similar to those from Virginia pine. An average of over 100 stunt nematodes per liter of soil and 18 propagules of *P. irregulare–P. debaryanum* per gram of soil were isolated. From an aggregate of 52 percent ectomycorrhizal development, less than 12 percent were formed by Pt in the VMI treatment. Pt was reisolated from nearly 50 percent of the surface-sterilized ectomycorrhizae. No Pt ectomycorrhizae were present on seedlings in the BI treatment. Total ectomycorrhizal development varied between 52 and 72 percent and

[3] Nematode extraction and identification by Dr. John L. Ruehle, USDA Forest Service, Forestry Sciences Laboratory, Athens, Georgia 30602.

TABLE 1. Growth and ectomycorrhizal development of three pine species after 8 mo in soil infested with Pisolithus tinctorius *(Pt) at Andrews Memorial Nursery, Chiefland, Florida. Each number is the mean of 30 seedlings, ten from each of three plots.*[1]

Inocula of *P. tinctorius*	Total plant fresh wt (gm)	Shoot-root ratio	Percent short roots ectomycorrhizal by		
			Pt	Other	Total
		LOBLOLLY PINE			
Vegetative mycelium	14.4a	2.0a	40a	32a	72a
Basidiospores	14.8a	2.15a	29b	46b	75a
None (control)	13.9a	2.23a	0	66c	66a
		SLASH PINE			
Vegetative mycelium	14.2a	2.94a	72a	10a	82a
Basidiospores	14.5a	2.92a	45b	43b	88a
None (control)	12.3a	2.96a	0	73c	73a
		SAND PINE (OCALA VARIETY)			
Vegetative mycelium	8.1a	3.77a	22a	18a	40a
Basidiospores	9.7a	3.85ab	4b	29a	33a
None (control)	6.1a	4.08b	0	21a	21a

[1] For a given pine species and measurement, means followed by a common letter are not significantly different at $P = 0.05$.

averaged 61 percent. Appreciable differences in soil chemical analyses among Pt treatments or pine species were not detected.

Andrews Nursery, Florida.—Seedlings of all pine species in the VMI-treated plots had ectomycorrhizae formed by Pt as early as 6 wk after emergence. Pt ectomycorrhizae were not detected on seedlings in the BI treatments until 3 mo after emergence. However, all seedlings examined had a few ectomycorrhizae formed by unidentified fungi as early as 6 wk after emergence. Basidiocarps of Pt were first observed in the VMI treatments of loblolly and slash pine plots in late August and in the sand pine plots in September. Production of basidiocarps continued until seedlings were lifted. Plots infested with Pt were examined only twice between August and November, the major period of basidiocarp production. However, totals of 78, 66, and 18 basidiocarps were collected from the VMI treatments in the loblolly, slash, and sand pine plots, respectively; and totals of 54, 39, and 6 basidiocarps were collected from the corresponding BI treatments. Most basidiocarps were between 1 and 6 cm in diameter and both stipitate and nonstipitate forms were observed within the same plot.

Significant growth differences due to Pt ectomycorrhizae were not detected, although seedlings with Pt seemed somewhat more vigorous. Significantly more ectomycorrhizae were formed by Pt on pine species from VMI than from BI inoculum. The aggregate percentage of short roots ectomycorrhizal on each species, however, was not affected by Pt treatment. Pt ectomycorrhizae from VMI significantly reduced the shoot-root ratio of sand pine (Table 1). Ectomycorrhizae formed by Pt on all pine species were morphologically similar to those previously reported (Marx and Bryan 1975). Pt was reisolated from over 40 percent of the surface-sterilized ectomycorrhizae. The ectomycorrhizae formed by other fungi were primarily bifurcate or simple coralloid and were cream-brown in color, similar to those formed by *Thelephora terrestris* (Marx and Bryan 1975).

Lateral spread (average of both directions at ends of each plot) of Pt from VMI and BI treatments into adjacent nonin-

TABLE 2. Growth and ectomycorrhizal development of three pine species after 6 mo in soil infested with Pisolithus tinctorius *(Pt) at Edwards State Nursery, Morganton, North Carolina. Each number is the mean of 30 seedlings, ten from each of three plots.*[1]

Inocula of *P. tinctorius*	Height (cm)	Stem dia (mm)	Fresh wt (gm) Shoot	Root	Total	Shoot-root ratio	Percent of short roots ectomycorrhizal by Pt	Other	Total
			LOBLOLLY PINE						
Vegetative mycelium	21.1a	4.2a	8.6a	3.9a	12.5a	2.21a	62a	2a	64a
Basidiospores	18.3b	3.8b	6.3b	3.3a	9.5b	1.91a	43b	25b	68a
None (control)	14.9c	3.3b	3.8c	1.4b	5.2c	2.71b	0	50c	50b
			VIRGINIA PINE						
Vegetative mycelium	17.0a	4.0a	11.5a	6.7a	18.3a	1.72a	68a	4a	72a
Basidiospores	13.8b	3.26b	7.1a	3.3b	10.5b	2.15ab	5b	53b	58b
None (control)	13.5b	2.7b	6.4a	2.5b	8.8b	2.56b	0	47b	47b
			WHITE PINE						
Vegetative mycelium	8.7a	1.8a	1.4a	1.8a	3.2a	0.78a	37a	10a	47a
Basidiospores	7.0b	1.5b	0.8b	1.0b	1.8b	0.80a	17b	10a	27b
None (control)	6.7b	1.5b	0.7b	0.9b	1.6b	0.78a	0	15a	15b

[1] For a given species and measurement, means followed by a common letter are not significantly different at $P = 0.05$.

fested soil was 80 and 40 cm in the loblolly pine plots, respectively. No *Pythium* spp. were isolated, but an average of 24, 3, and 9 stubby-root (*Trichodorus* spp.) nematodes per liter of soil were extracted from the loblolly, slash, and sand pine plots, respectively. Pt treatment did not significantly affect nematode populations.

Significant differences were not detected in chemical soil analyses among treatments, although the quantity of most major nutrients was consistently lower in soil infested with VMI of Pt.

Edwards Nursery, North Carolina.—Six days after the experiment was installed this nursery was flooded by 10 cm of rainfall in a 36-hr period. The beds remained under water 4 days and the majority of the sawdust mulch and large quantities of seed were washed from the plots. This caused erratic seed germination and a highly variable seedling density within and between plots.

Ectomycorrhizae formed by Pt from VMI were extensively developed on all pine species as early as 9 wk after seedling emergence. Ectomycorrhizae from BI were detected after 12 wk. Seedlings in the NI plots had a few ectomycorrhizae formed by unidentified fungi after 9 wk.

Basidiocarps of Pt were first detected in the VMI plots of Virginia and loblolly pines in early August. Basidiocarps in the white pine plots of the VMI treatment were not observed until late September. This was also the time basidiocarps were first detected in the BI treatments of the Virginia and loblolly pines. No basidiocarps were observed in the BI-white pine treatment. Totals of 51, 63, and 24 basidiocarps were collected from the VMI treatments in loblolly, Virginia, and white pine plots, respectively; and totals of 27, 33, and 0 basidiocarps were collected from plots with the BI treatments. Some of the basidiocarps, especially in the loblolly and Virginia pine plots of the VMI treatments, were over 12 cm in diameter and 18 cm tall. Both stipitate and nonstipitate forms of Pt were observed.

Growth measurements (height, stem diameter, and shoot and root fresh weights) of seedlings due to Pt ectomycorrhizae were significant. Fresh weights of shoots plus roots of seedlings in VMI plots in com-

parison to NI seedlings were 140, 107, and 100 percent greater on loblolly, Virginia, and white pines, respectively (Table 2). Infestation of soil with Pt significantly increased total ectomycorrhizal development on seedlings of all pine species. There was a direct correlation between amount of Pt ectomycorrhizae and growth stimulation of seedlings. Pt ectomycorrhizae on loblolly pine seedlings from BI stimulated growth by over 80 percent. Considerably more ectomycorrhizae were formed from VMI than from BI on all pine species. There were also significant reductions in shoot-root ratios of loblolly and Virginia pine seedlings due to Pt. Seedling density in the plots was not influenced by Pt treatment. The ectomycorrhizae formed by Pt on loblolly and Virginia pines were identical to those described previously (Marx and Bryan 1975). Those formed on white pine, however, were not as elongated or as multibranched as those on the other pine species. All Pt ectomycorrhizae had extensive hypal strand development. Pt was reisolated from over 55 percent of the surface-sterilized ectomycorrhizae of all pine species. Lateral spread (average of both directions at ends of each plot) of Pt from VMI and BI treatments into adjacent noninfested soil was 65 and 50 cm in the loblolly pine plots, 43 and 8 cm in the Virginia pine plots, and 60 and 50 cm in the white pine plots, respectively. Pt treatment did not significantly influence the number of propagules of *Pythiums* isolated from soil. Density of *P. irregulare–P. deharyanum* averaged 13 and 6 propagules per gram of soil in the loblolly and Virginia pine plots; none were detected in the white pine plots. Plant-parasitic nematodes were not found in this nursery. Differences were detected in soil nutrients between plots of the different pine species, but not between treatments of Pt.

Discussion

Effective soil fumigation must precede successful artificial soil infestation with either vegetative or basidiospore inocula of Pt. High soil populations of *Pythium* spp. and plant-parasitic nematodes and the early appearance of *Thelephora* basidiocarps indicate that soil fumigation was ineffective in the Georgia nursery plots. This was probably due to cool soil temperature and high soil moisture at the time of fumigation. Correlated with these microbial populations was the near total failure of either form of inocula of Pt to colonize seedling roots successfully.

Undoubtedly other soil factors influence successful infestation of soil with Pt, but they appear to be less important than effective soil fumigation. Type of mulch, quantity of mineral spirits used for weed control, seed treatment, ferbam for fusiform rust control, and previous cropping histories do not appear to be limiting factors in the artificial establishment of Pt in soil. Most of the uncontrolled variables were either common in all nurseries, or occurred in one of the nurseries with successful establishment of Pt (Florida or North Carolina) and in the unsuccessful experiment in Georgia.

In the Florida nursery, Pt did not dominate seedling roots or stimulate seedling growth as it did in the North Carolina nursery and in our previous test (Marx and Bryan 1975) with vegetative mycelium inoculum. Perhaps fumigation at the Florida nursery was not totally effective. All indigenous fungal symbionts may not have been eradicated or colonization of effectively fumigated soil by airborne inoculum of naturally occurring ectomycorrhizal fungi may have been extremely efficient. Both of these possibilities are supported by our observations of ectomycorrhizal development on seedlings in all plots in the Florida nursery as early as 6 wk after emergence. This was about 3 wk earlier than in the North Carolina nursery. Early ectomycorrhizal development by other fungal symbionts may be directly competitive to establishment of Pt on the roots, or other soil organisms may compete with Pt in the rhizosphere.

In spite of the flooding that occurred in the North Carolina nursery shortly after installation of the experiment, colonization of roots by vegetative inoculum of Pt was excellent. The resulting growth stimulation

of seedlings demonstrated the potential value of Pt in improving seedling quality. The lower incidence of Pt ectomycorrhizae from basidiospore inoculum on all pine species and the late appearance of Pt ectomycorrhizae on these seedlings indicates that it takes basidiospore inoculum longer than vegetative inoculum to colonize roots. During this delay in ectomycorrhizal synthesis, naturally occurring symbiotic fungi may colonize the soil and compete with Pt for feeder roots, resulting in a mixture of ectomycorrhizal fungi on roots of seedlings by the end of the growing season.

The ability of Pt to spread laterally in soil along the longitudinal axis of the nursery beds from its site of original introduction has practical significance. Total lateral spread (total longitudinal spread into originally noninfested soil on both long axes of the original plots) averaged for all pine species in the Florida and North Carolina nurseries was nearly 120 cm from vegetative inoculum and over 50 cm from basidiospore inoculum. Thus, after one growing season, the lateral spread from vegetative inoculum had nearly doubled and lateral spread from basidiospore inoculum increased by nearly half the total soil volume containing Pt.

Marx and Bryan (1975) reported that Pt survived overwintering in soil after seedling removal in the fall. When overwintered, the infested soil was diluted 1 : 5 with freshly fumigated soil, and seedlings grown in the diluted soil became heavily ectomycorrhizal with Pt. Theoretically, a 10-fold increase of Pt-infested soil in a nursery could be achieved in one year if a doubling of initially infested soil volume were to occur during the growing season and this volume were to be mixed 1 : 5 with freshly fumigated soil for seeding the next crop. Care must be taken, however, to avoid spreading pathogens and other pests with this practice.

Production of basidiocarps of Pt from introduced inoculum has significance, especially as a source of new inoculum in the nursery. Basidiospores can be easily collected and stored (Marx 1975) and then used to infest more soil, or they could disseminate from basidiocarps via natural means (air, water, insects, movement of soil) to colonize other nursery areas. Either means of dissemination should increase the incidence of Pt on seedlings in a nursery. The seedlings, however, would undoubtedly also have ectomycorrhizae formed by other fungi. The quantity of Pt ectomycorrhizae would be regulated by the competition of the other naturally occurring fungi.

Literature Cited

HACSKAYLO, E., and J. A. VOZZO. 1971. Inoculation of *Pinus caribaea* with ectomycorrhizal fungi in Puerto Rico. Forest Sci 17:239.

HENDRIX, F. F., JR., and W. A. CAMPBELL. 1973. Pythiums as plant pathogens. Ann Rev Phytopathol 11:77–98.

———, and E. G. KUHLMAN. 1965. Factors affecting direct recovery of *Phytophthora cinnamomi* from soil. Phytopathology 55:1183–1187.

JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis Rep 48:692.

LAMB, R. J., and B. N. RICHARDS. 1974. Inoculation of pines with mycorrhizal fungi in natural soils. I. Effects of density and time of application of inoculum and phosphorus amendment on mycorrhizal infection. Soil Biol Biochem 6:167–171.

MARX, D. H. 1973. Growth of ectomycorrhizal and nonmycorrhizal shortleaf pine seedlings in soil with *Phytophthora cinnamomi*. Phytopathology 63:18–23.

———. 1976. Synthesis of ectomycorrhizae on loblolly pine seedlings with basidiospores of *Pisolithus tinctorius*. Forest Sci 22:13–20.

———, and W. C. BRYAN. 1971. Influence of ectomycorrhizae on survival and growth of aseptic seedlings of loblolly pine at high temperature. Forest Sci 17:37–41.

———, and W. C. BRYAN. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. Forest Sci 21:245–254.

———, W. C. BRYAN, and C. B. DAVEY. 1970a. Influence of temperature on aseptic synthesis of ectomycorrhizae by *Thelephora terrestris* and *Pisolithus tinctorius* on loblolly pine. Forest Sci 16:424–431.

———, W. C. BRYAN, and L. F. GRAND. 1970b. Colonization, isolation, and cultural descrip-

tions of *Thelephora terrestris* and other ectomycorrhizal fungi of shortleaf pine seedlings grown in fumigated soil. Can J. Bot 48:207–211.

MOSER, M. 1958. Die kunstliche Mykorrhizaimpfung von Forstpflanzen. II. Die Torfstreukultur von Mykorrhizapilzen. Forstwiss Zentralbl 77:257–320.

———. 1959. Die kunstliche Mykorrhizaimpfung von Forstpflanzen. III. Die Impfmethodic im Forstgarten. Forstwiss Zentralbl 78:193–202.

SCHRAMM, J. R. 1966. Plant colonization studies on black wastes from anthracite mining in Pennsylvania. Amer Phil Soc Trans 56: 1–194.

SHEMAKHANOVA, N. M. 1967. Mycotrophy of woody plants. Isr. Program. Sci. Transl. Jerusalem. (Original in Russian, Izd, Akad. Nauk. SSSR Moscow, 1962.) 329 p.

THEODOROU, C. 1971. Introduction of mycorrhizal fungi into soil by spore inoculation of seed. Aust Forest 35:23–26.

———, and G. D. BOWEN. 1970. Mycorrhizal responses of radiata pine in experiments with different fungi. Aust Forest 34:183.